Title: What are the links between antibiotic usage and resistance in animals and people?

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- Hello everyone, and welcome to my talk, what are the links between antibiotic usage and resistance in animals and people? My name is Tamzin Dewé, I'm the Head of Evidence in the AMR team at the Veterinary Medicines Directorate. Now my background, I'm a vet, I'm also an infectious disease epidemiologist, and I almost have a PhD in this subject in which I'm about to speak, specifically modelling the links between antibiotic usage and resistance. So the learning objectives. On completion of this module, participants will be able to explain the key features of bacterial population dynamics and the evolution of resistance. Summarise the evidence linking antimicrobial usage and antimicrobial resistance in animals and people, and describe trends in UK AMR data.

Now, we'll just say the one line, gonna constantly refer to antimicrobial usage and resistance in this presentation. I am only talking exclusively about antibiotics here not some of the other types of antimicrobials. So to start with, we're going to cover some of the important concepts of bacterial population dynamics, specifically clones and lineages, competition, host adaptation and then the horizontal gene transfer or HGT. Okay, so, sorry, that's my laser pointer. The first of these concepts is a clone or lineage. Now clone is a collection of bacteria that were centred from a single common ant system. I'm gonna give you an example of how clone arises. So here we have here, two bacterial cells, the green and the orange guys there. They belong to the same species but they differ in some meaningful genetic way. And so those cells are busy multiplying, multiplying, multiply, multiply, reproducing. And what we have here are two different clones. So a clone of the orange bacteria and a clone of the green bacteria. Now, if some of those green bacteria mutate to blue and then reproduce, we now have kind of two additional clones, one being this large clone of the green and the blue bacteria, all right? 'Cause remember they all descend from the same common ancestor, this guy over here, but we also have a smaller clone, which is the blue cell.

So there you go. A clone is a bunch of bacteria descended from a single common ancestor. Okay, so now we're gonna talk about competition. Let's think of these different clones or strains of the same bacterial species existing now within a host niche that the black box here. Now, this niche could be an individual host or it could be across a whole population. For example, the gastrointestinal tract of a flock of poultry. And those different strains existing within that niche are currently competing against each other for host resources. So let's imagine them as kind of filling that niche if you like, shown by the different colours here. Now different strains will expand and decline over time, I'm talking months to years usually, depending on which strain is the most fit at a given point in time. And so you can anticipate that competition it's kind of constantly happening between these different strains. And you can anticipate that in a host niche that is getting exposed to antibiotics, those lineages which are resistant will be fitter overall, right? They've got an advantage of being resistant to the bacteria that's killing the susceptible, the other susceptible strains. And they're gonna be able to expand within that host niche.

Okay, the next concept I want to cover is host adaptation. We're gonna talk about what happens when there are multiple hosts niches, or host populations. Shown here by my stylish icons, I will just say there's nothing specific or particular about pigs. I'm not trying to single out pig production or pig vets, it was just a nice icon. The key point here is that we're talking about two different host species. Okay, now both of these host population shown here have their own native population of bacteria shown by the coloured dots. And transfer of bacteria between different hosts occurs relatively frequently, right? So we've got the pig adaptive bacteria coming into contact and affecting a person, and likewise can happen in opposite direction. Now whether that new infection is self-limiting, or it's followed by onwards transmission and expansion of a clone in the new host species depends on the extent of hosts adaptation. All right. So are we going to see the bacteria just kind of staying in that first person that it infects or is it going to be sufficiently adapted to the new host population that it can spread further, like what's happening with the orange cells here. And the ability of those bacteria to do that, to adapt the new host species depends on the bacteria themselves.

Okay, now I'm gonna show you some evidence for some of the examples of the different ways that host adaptation happens for different bacterial species. What we're looking at here, three phylogenetic trees. Now, a phylogenetic tree is a diagram that indicates the genetic relatedness of different bacterial isolates. So I should just explain to you how to read these. So each dot represents a different bacterial isolate. So a specific individual genome. And how we read these trees is we trace the branch, the black branch coming out of the isolate back to where it attaches to other branches, okay? And this point of connection is called a node. And that node represents the most recent common ancestor of all the isolates that are stemming from that node, okay? So what we can see here is how closely together different isolates from different host species are clustered together. So what we're looking at with E. coli are multiple generalists lineages in this tree. So you can see each different colour of these isolates indicates the animal or person from which they were sampled. So what we're seeing here is a bunch of mostly chicken and human isolates that are clustered together or grouped together, right?

By contrast, when we look at the Campylobacter to Jejuni tree, while we are seeing some generalist lineages, right? So here's a mixed cluster, okay? Of isolates from different host species. We're also seeing some host specific lineages like this one here, okay? Everything there, almost everything there is bacteria that's been isolated from chickens but also these open circles, also a couple of trans infections from people that like what might occur through a food borne infection. And then finally the staff orients which is quite a different tree structure. Again here we're seeing mostly clustering of host specific lineages, right? So you've got a bunch of red ones here, a bunch of red ones here, a bunch of yellow ones there okay? So they're indicating host adopted lineages. However, we do have a few examples of more varied lineages there as well.

Okay, so the next concept to get familiar with is horizontal gene transfer. So here's a bacterial cell, it's got its chromosome, and it's also got a plasmid which is a chunk of extra chromosomal DNA. Now when that bacterial cell multiplies and expands we're seeing vertical gene transfer, right? Where that DNA is being passed on to all the descendants of that original bacterial cell. Now a really key feature of bacteria is that it can also undergo horizontal gene transfer, which is when genetic material is transferred horizontally between unrelated cells, okay? So that can occur via plasmids, but it can also occur via other chunks of DNA including on the bacterial chromosome which are also able to do the same thing and kind of jump across to it, to an unrelated cell. And all these chunks of DNA together, are called mobile genetic elements. And they're really, really important because once that transfer has happened, so we've got transfer here of this pink plasmid from the yellow cell to

the green cell, when that green cell reproduces that plasmid is then conveyed to all the descendants of that green cell as well, okay?

Now, as I said, it's a really, really frequent mechanism for transferring new chunks of DNA, to unrelated cells from a different bacteria strain, or even a different species. And this is particularly important for resistance because resistance genes are frequently found on mobile genetic elements, such as plasmids. I've shown you this slide before to demonstrate bacterial transmission between hosts, but I just wanted to go into how horizontal gene transfer can impact this process as well. So here, we've got the yummy bacteria jumping over into the human host population, and then there's there's clonal expansion. Now the first thing to recognise is that horizontal gene transfer can facilitate that process in itself, okay? For example by transferring genes for host adaptation. So that yellow cells dumped across into the human population, it's not really well adapted to the human hosts, and maybe would not necessarily expand, but through horizontal gene transfer, it's able to pick up additional genes that help it reproduce better in the human host.

Now, so that's one important feature of HDT when it comes to your host adaptation. But another important feature is that host adaptation doesn't even have to occur for genetic material to be introduced into that new host population and to spread. So here's an example. We've got this pig, sorry, pigs again, infected with green bacteria. And we have infection of a new host, the person here, with this green bacteria. Now this is a dead end infection, okay. The green bacteria is not hosted up to it, it hasn't managed to pick up any hosts adaptation genes through horizontal gene transfer. So this person's gonna get sick and then they're gonna get better and that's it. However, if horizontal gene transfer of that plasmid occurs into a different bacterial species into the blue bacteria, which is already resident in the human population, already adapted to the human population, that we don't actually even need host adaptation of the green bacteria to occur for the genes on that plasmid to spread within the human population, okay. So clonal expansions of blue bacteria, which is already adapted to people can happen, again just carry that, the genes on that plasmid through that population. So again, a really, really key way for new, for genes to be introduced to a new host population. And those genes can include resistance genes, okay? So again, just coming down to why horizontal gene transfer is so critical when, particularly when we're talking about jumping the species barrier.

Okay, here's another study, an example from another study to give you some evidence of how this works. So what we're looking at here is a network analysis of the staff aureus accessory genome. Now the accessory genome is everything that's not the core genome of a bacterial species. The core genome being those genes that are shared between every isolate of that species. So really kind of the core, of what makes a staff aureus a staff aureus. And the accessory genome is everything else. So it's, includes mobile genetic elements and it's things that are kind of optional extras if you like. So this network analysis of the accessory genome what it's showing you is that we have clustering of the accessory genome in different host species. So I've got a bunch of red genomes here, bunch of pink here, associated with pigs, and a bunch of horse associated genomes here as well. So what that's showing you is that those genes in the accessory genome, so the mobile genetic elements are really really critical for host adaptation. We're still seeing some clustering of the room in this isolates, but there's just a bit more diversity there within the room in a population and also within the light blue ones, which are people. And so again, this is indicating because we're seeing this clustering of the accessory genome, this is indicating that HGT is important for host adaptation.

Okay so those are all the key concepts, I hope that wasn't too much. We looked at bacterial clones and lineages, how competition works, how host adaptation works. And then we touched on horizontal gene transfer and its implications there. So, now I'm gonna move on to the second part of

this lecture, which is about the evidence linking antimicrobial usage and antimicrobial resistance. Now, there really is now a wealth of evidence demonstrating that AMU drives AMR at multiple levels. This has been shown within individuals, humans and animals within farms, within communities, and at regional national level as well. And the main reason for why this is such a successful driver of resistance is because there's usually in almost all cases, there's a fitness cost associated with resistance. So that means that for a bacterial cell to be resistant, so for it to generate the enzyme that allows it to break down antibiotics, or fabricate other proteins that allow it to resist those antibiotics, that costs energy to the bacterial cell right? It uses up resources of that cell that can then not be diverted for other purposes, for example, growth and reproduction. So that's why we see this consistent pattern of AMU driving AMR. Now on the other on the side, we now have more compelling evidence that reducing AMU also reduces AMR. And the most interesting paper that I'd like to talk to you about is this one by Karen Tang and colleagues which is a systematic review and a meta analysis of interventions to reduce AMU in animals. And what I did is I looked at a bunch of different studies over 80 different studies that had a bunch of different interventions to reduce antimicrobial usage across different host species, different timeframes. And then I measured, these studies had then measured resistance after these interventions. And what Tang and her colleagues have done, is that they've then poured the results of those studies across different bacterial species, sample type. So where the sample's taken from food or from faeces, for example, and antibiotics. And what they showed was that almost all combinations of bacterial species, antibiotic and sample type showed a reduction in AMR. Now the range of that reduction varied between one and 39% but most of the studies demonstrated a 10 to 15% reduction. The greatest effect was for enterococcus, and there was less for some of the other species, and there was a greater affection for pigs and poultry compared to cattle. Now these numbers, however, are not in themselves very meaningful because the authors haven't corrected for the size of the reduction AMU, okay? So they haven't actually done any kind of comparison of the percentage reduction in AMU and how that impacted the AMR. They've just stuck everything together into that meta analysis. But what's really compelling about the results is that the overall effect of the interventions, irrespective of the amount that AMU was reduced is that there was overwhelming reduction in AMR, across that kind of vast range of different host species different interventions, different time periods. So this is actually really, really compelling evidence. And it's a body of evidence showing us that yes, when we reduce antimicrobial consumption we should see a reduction in AMR.

Okay, so now we're gonna move on to the links between AMR in animals and AMR in people. Now, genomic studies do indicate that the impact of AMR in animals or AMR in people varies widely, usually by bacterial species. But there are other factors at play as well. I'm just gonna show you some of the examples of the patterns, the different patterns of AMR spread between animals and people. So in clostridium difficile for example, we see bi-directional spread between humans and animals and a collection of AMR genes that are not so specific, okay. So there's constant back and forth there in terms of infection and exchange of AMR genes. By contrast, in livestock associated MRSA, we see quite different pattern which is a host species jump, and then onwards transmission of AMR. So and that's the phylogeny that's shown here on the right. Now don't be put off like being in a circle, it's still showing you exactly the same thing, it's just how the authors want to present it. So what we're looking at here, human associated isolates, the yellow and the red, and pig associated isolates of LA MRSA, which are the green boxes. And you can see there are multiple instances where you have a bunch of isolates from people, okay, all these yellow dots here, and they're closely associated with an isolate from pigs. So what we're seeing there is an example of transmission of this bacteria and it's resistant genes from pigs to people. And then we're actually having onwards transmission within the human population as well.

Okay, so for some other bacterial species and the pictures are slightly less clear cut than that. I'm gonna show you here some examples of bacterial species where we have different lineages and resistant profiles circulating largely separately in human animal hosts, but with some spillover between those two populations, the first example I'm gonna show you is a Salmonella Typhimurium epidemic that's linked to this phylogenetic tree here. And what we're looking at, what we're looking at here is clustering of the human and the animal isolates separately, okay? So these red isolates here accosted together and all these blue isolates that are over here accosted together, okay. So we've largely got kind of separate circulation of those isolates. However, there are some examples where cross-species transmission occurs. Here where we've got a branch changing from blue to red, and then there's some examples over here, yet red to blue, okay. So largely host species specific but some spillover, and this is consistent with what's being shown in these Venn diagrams on the right. So the top of these Venn diagrams is showing you the different resistance genes circulating in human-animal populations. You can see there's quite a large overlap there. When we look at the bottom Venn diagram, which represents the resistance profiles, so the different combinations of resistance genes that might be present in a single isolate, you can see that there's much, much less overlap, okay? So again, it's giving us this picture of largely separate, but some crossover, okay. And the same is true for extended-spectrum beta-lactamase producing E. coli in the UK. Now extended-spectrum beta-lactamases or ESBLs allow breakdown of cephalosporins, which is a critically important antibiotic in human medicine. And this paper by day, they show a similar pattern that most human infections are due to human associated lineages of this bacteria, but animal lineages are contributing, but they're not constituting the majority of human E. coli infections.

Now that said, it gets more complicated because that picture can differ for different lineages of those bacterial species. So there are some examples where we actually have frequent horizontal gene transfer, including of AMR genes between isolates from animals and people. Some examples are Salmonella Newport in the US, we've got lots of overlap in the AMR genes in human and in bovine isolates, and a particular lineage of E. coli which is showing a large overlap between avian pathogenic and human extraintestinal isolates. And that's actually what I've got here on the right. So this is a phylogenetic tree again of the accessory genome. So mobile genetic elements and including resistance strains. What we see here is all these blue ones, right? Which are human associated clade and these green ones which are all from people as well okay? But the rest of the tree we're seeing kind of, what's the word? Interspersed, sorry interspersed between each other we're seeing these isolates are coming from people which is the black, and then the isolates from poultry which are the red dots, okay? So this is a more complicated picture.

I've just told you that equalise largely circulating in kind of human adapted and animal adopted strains. But for some lineages, we have much more crossover, and that includes of the AMR genes as well. Campylobacter is example of another one that shows frequent interchange between human and animal isolates and in that case with the environment as well. So to summarise, what I wanted to say to you about AMR between animal and people, the transmission of AMR between animals and people, is that gene exchange between bacteria happens all the time, and it does happen across the host species barrier. But the magnitude of the impact of animal AMR on human AMR varies widely. And this depends on the bacterial species or as I've just shown you even the lineage, but also the natural history of the bacteria and the particular environment in which it's existing. So just to give you an example, for gastrointestinal infections that are usually self-limiting in people, for example Campylobacter foodborne transmission is a major contributor to disease in people. Whereas for E. coli, which has several human adapted strains the magnitude of the animal attribution to the AMR burden in people is less. However, I do wanna just underline that all roots represent a risk of introduction of AMR genes into human bacterial populations, and clonal

expansion of that resistance within people, because of how effective HDT is at introducing new genetic material across species barriers.

Okay, so now I'm gonna take you through some of the statistical evidence, looking at the associations between AMU and AMR in animals and people. This report is called the JIACRA report. This is the second one of these reports that's come out. It's based on the data from the EU harmonised monitoring for AMR, which we, until recently contributed to. So what this report is, is that it looks at year by year study school associations between AMU and AMR in animals and people. And so I'm gonna go through some of the examples of their results. Now, what they did find was that the strength of associations between AMU and AMR varied by bug drug combination. Again, what we would expect based on the evidence that I've already shown you in terms of the kind of species differences. What they did find, however, was that consumption of fluoroquinolones or third generation cephalosporins by people impacted resistance in E. coli in people. Again, that's consistent with the genomic information about how those lineages circulate in the different host populations. However, they do indicate that consumption of fluoroquinolones and other quinolones by food producing animals impact Salmonella and Campylobacter Jejuni resistance, and this intern impacts resistance in people. And the same association was found for tetracycline consumption and resistance in Campylobacter

Now, I'm gonna go back to the Tang meta analysis which also looked at the effect of interventions to reduce AMU in animals, on the prevalence of AMR in people. Now in this case we are able to include far fewer studies and then for the animal only analysis. And they were predominantly dominated by poultry and pig farms and were confined to bacterial species. However, again, in that case, we saw a significant or substantial reduction in the prevalence of AMR in people after consumption by animals was reduced. Now I'll say again, that the number of studies here was fairly limited and they were mostly dominated by research looking at the prevalence of AMR in farm workers, okay? So it doesn't necessarily translate to the wider community, okay? It also does not include important foodborne pathogens. However, it's still a significant piece of evidence indicating to us concretely that interventions to reduce antimicrobial use can result in the AMR burden in people decreasing. So, and here's another study that was picked up by another systematic review, similarly looking at interventions. This study measured the prevalence of resistance in Salmonella Heidelberg that was isolated from retail chicken and clinical samples from people. And what they did is they looked at the impact of removing in-ovo safety a fewer injections in broiler chickens. And you can see here, so I'll just get a little pointer, after this antibiotic use was ceased they had a fairly prompt and very substantial decline in the prevalence of resistance in retail chicken, but also a big decline in the prevalence of resistance in, by that species in, sorry by Salmonella Heidelberg in human clinical isolates. And then interestingly after there was a partial re-introduction of the safety of fewer use, they started to see an uptick in the prevalence of resistant infections in people.

Okay, to summarise, everything I've told you so far about the links between AMU and AMR. Now we know very clearly that AMU drives AMR. We also know that interventions to reduce AMU should or almost always do result in reduced AMR. That might not happen immediately, it might take a few years, but it should happen. And as I said, almost always happens. Now, there are some exceptions, these include when there is co-selection of resistance and this was something that was actually observed in the EU after Abbey Parson was bound as a growth promotion in the 90s. And when that happened, there was, in some sectors and in some countries, there was a really strong reduction in vancomycin resistance in enterococci. However, it wasn't seen in all sectors and in all countries. And after further investigation was done, and over time, the realisation was made that the reason for AMI not reducing in those instances was because there was co-selection occurring. So there was

some other antibiotic that was still being used in that population, that was genetically linked to the resistance to vancomycin. And so it was actually co-selection to that additional antibiotic that was driving or maintaining the prevalence of resistance in those cases. So in that case, what was required to reduce the prevalence of AMR, was not just a reduction in the one antibiotic but reduction in additional antibiotics as well. Now, another exception to this kind of general rule is when there's expansion of a clone that's fit for other reasons. So it's a bacterial clone that is resistant but it's also more fit compared to some susceptible strains for other reasons. And so you can imagine in that case, even if anti-microbial consumption is reduced that coin's still kind of happily expanding and taking over if you like, maybe not taking over but expanding in the population, irrespective of antibiotic consumption, doesn't need it. Now that's fairly rare, although we do think it's something that's happening in the UK at the moment, and I'll touch on that in a minute.

Now, what we also know about AMU and AMR is that these links are more difficult to measure when we're crossing the barrier between different host species. And the extent to which animal associated bacteria or AMR genes contribute to the human burden does depend on the organism. Okay, so the final section of this lecture is just to tell you a little bit about some of the AMR results that we have from the UK. So to start with, we're gonna look at E. coli in broilers, this data is taken from our UK-VARSS report. And what we're seeing here, over the last few years has been a substantial reductions in the prevalence of resistance to a number of commonly used antibiotics in this sector. And we've also seen a reduction to Ciprofloxacin which is a highest priority critically important antibiotic for human use. Now these declines are not the same as what's being seen in many other European countries, okay? And it is reflective of, it is reflective of the substantial declines in antibiotics that's happened in this sector, okay? So here, we've got vast reduction in the amount of antibiotics that were used, that reduction is not happening in every European country and their AMR results reflect that. Okay, so now we're gonna look at resistance in E. coli from pigs. Again, what we've seen over time is a reduction in resistance to a number of commonly used antibiotics. We are however seeing, perhaps something of an uptick in resistance to ampicillin and trimethoprim, I don't know if that's just a kind of fluctuation or whether that's a new trend, which would be concerning given that consumption of both these antibiotics, sorry, was penicillin here, the pink one, and trimethyl and sulphonamides has decreased substantially in this sector as well and continues to decrease. And one thing that's interesting is I noticed that consumption of kind of the ABO group of antibiotics has increased slightly in that time. Is the coast selection going on there that's driving the resistance here? Don't know we just don't have enough information. It'll be interesting to see what comes out of the data in this year in particular. And we'll also say it's not shown here, but the resistance to highest priority, critically important antibiotics in this species is very, very low, which is great.

So now I'm gonna show you the kind of overall results for indicator E. coli in pigs and poultry. What we have here on the left is a graph looking at the prevalence of E. coli isolates weighted by the biomass of that species. And two of the important trends that I want to pick out is we're seeing an increase in fully susceptible E. coli, and a decrease in the ESBL producing E. coli. So again, remember, these ESBLs are able to break, ESBL producing E. coli are able to break down cephalosporins. Now this is not what's happening in the human population, okay. Over here, we have these brown sections of the bar, is the number of resistant bloodstream infections in people. And the prevalence resistance is shown here in black. And you can see that's actually increasing. Now this is reflective of a general trend that's happening in people at the moment in the UK, which is broadly an increase in the prevalence of resistance infections. So this is consistent with some of the information I showed you earlier, which indicates that ESBL, E. Coli, and E. coli generally are largely adapted to people and they're kind of circulating doing their own thing. However, what's important to point out here is that

we still don't know actually what proportion of those human bloodstream infections are actually attributable to animals, we just don't have that data.

Okay, so that was the picture in E. coli. And you can see that it's pretty much positive in terms of the links between AMR in that bacteria in animals and people in the UK. Now I'm gonna look at Campylobacter which unfortunately is not nearly so encouraging a picture. And what we're seeing in Campylobacter over here on the left is persistently high and increasing prevalence of resistance to Ciprofloxacin and Tetracyclines in particular. Now this is concerning because Ciprofloxacin is again a highest priority, critically important antibiotic. It's really, really important for human medicine. And what's also happening is that, is that prevalence of resistance that we're seeing in broilers, is also feeding through to the prevalence of resistance in that species in both retail meat and people. I'm just gonna get my little pointer. So here, these red dots indicate recent data. So here we've got consistently high resistance in broilers translating to consistently high resistance in retail meat, translating to consistently high resistance in people. And I will just say that this is despite enormous reductions in fluoroquinolone use by the meat poultry sector. And it's also contradictory to the statistical associations that have been indicated Jack. Now remember I said that it very clearly showed there that for fluoroquinolone use in broilers was associated with antimicrobial resistance. So we're seeing a divergence there from what we would expect. Now, this is perhaps a bit of a complicated picture particularly because Campylobacter has a highly plastic genome it changes a lot. And also fluoroquinolones resistance in this species fairly unusually is not usually carried on a mobile genetic element, it's actually a result of a single point mutation in the chromosome. We also think that it's not associated with any fitness cost, again, unusually. So, there's perhaps not really any or very low negative impacts in this species to have resistance of fluoroquinolones. There's something else going on here as well which is the expansion of resistant clones we know from work done by Taylor, who's a PhD student, who's partly funded by the BMD, that, sorry I just wanna get my, yeah, there you go, that the prevalence resistance in this species is dominated by three highly resistant clones.So this is, you can see that these three clones circled here all have a prevalence of resistance above 70%. And that's, we think that that might actually be what's driving this persistently high fluoroquinolone resistance in this species. So yeah, we don't have the complete picture, it is concerning in that case.

So to summarise some of these results from AMR in the UK I keep clapping my hands, apologies everyone, hope that's not too loud, coming through my headphones. So what we are seeing is in many cases a downwards trend in AMR in the UK, okay? That's resulting from substantial reductions in AMU. It's working, please keep going, it's happening. Now, as I indicated earlier, that's absolutely what we would expect. And so there are some findings that need further investigation, particularly by genomic analysis, to try and more explicitly understand what's going on, in the cases where the findings are unexpected. And it is also something that we'll need to closely monitor, now that AMU seems to have plateaued, or that reduction in AMU seems to have plateaued. Of course, we do need to do additional research and we do need to do additional surveillance to fill in some of these evidence gaps, and particularly across the animal-human interface.

So that's it. I hope that wasn't too fast. I hope that you should now be able to sort of achieve these objectives. So explaining the key features of bacterial population dynamics, and the evolution of resistance. Summarise the evidence, linking AMU and AMR in animals and people, and also describe trends in UK AMR data. Here's a slide for some further reading. Please do have a look at the UK-VARSS Report, we'll have a new one coming out at the end of this year. And also the UK One Health Report, which is being led by the BMD this year, and we're hoping for publication in early 2022. If you go to read one scientific paper off the back of this talk, please do read the Tang matter analysis

on the links between AMU and AMR, it's really compelling, and it's an interesting accessible rate. So it's a bit statsy, but not overwhelmingly so, and I hope that you'll find it compelling and interesting. And there's a couple of other resources that you can have a look out there as well. And finally, I'm just gonna leave you with my references slide. If you want to look into anything in any more detail. And I just want to say thank you to everyone for listening. I hope it was interesting. I hope it was useful and keep up the great work. Thank you so much.